

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/70, 31/505	A1	(11) International Publication Number: WO 98/57651 (43) International Publication Date: 23 December 1998 (23.12.98)
<p>(21) International Application Number: PCT/US98/12717</p> <p>(22) International Filing Date: 18 June 1998 (18.06.98)</p> <p>(30) Priority Data: 60/050,031 18 June 1997 (18.06.97) US</p> <p>(71) Applicant (for all designated States except US): DISCOVERY THERAPEUTICS, INC. [US/US]; Suite E-17, 2028 Dabney Road, Richmond, VA 23230-3311 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): MARTIN, Pauline, L. [US/US]; 2302 Leighton Court, Richmond, VA 23233 (US). MCAFEE, Donald, A. [US/US]; 13408 Favero Court, Richmond, VA 23233 (US).</p> <p>(74) Agents: ESMOND, Robert, W. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).</p>	<p>(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: COMPOSITIONS AND METHODS FOR PREVENTING RESTENOSIS FOLLOWING REVASCULARIZATION PROCEDURES</p> <p>(57) Abstract</p> <p>In the present invention, a method is provided which reduces or prevents restenosis following revascularization procedures. It has now been found that selective stimulation of adenosine A_{2A} receptors can reduce or prevent such restenosis. This method may be achieved either by: (a) the administration of selective adenosine A_{2A} receptor agonists, (b) the administration of a selective adenosine A₁ antagonist in combination with either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, or (c) the administration of a selective adenosine A₁ antagonist in order to block adenosine A₁ receptor activation by endogenously-released adenosine. The present invention is also directed to an improved surgical procedure that relies upon selective stimulation of adenosine A_{2A} receptors.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Compositions and Methods for Preventing Restenosis Following Revascularization Procedures

Background of the Invention

Field of the Invention

5 The present invention relates to adenosine pharmacology and improved surgical procedures. More particularly, the invention relates to reducing or preventing restenosis following a revascularization procedure by selective activation of adenosine A_{2A} receptors.

Related Art

10 Occlusion of blood vessels can be treated with revascularization procedures that attempt to either remove the occlusion or to reroute blood flow through a bypass graft. A variety of surgical approaches have been used, including percutaneous transluminal coronary angioplasty (PTCA), directional coronary atherectomy (DCA) and excimer laser angioplasty. In order to provide
15 improved blood flow in the heart, for example, the insertion of coronary artery bypass grafts has become common practice. PTCA is an alternative procedure that is also conducted in patients whose coronary blood flow is severely impaired by the presence of atherosclerotic plaques within the coronary blood vessels. In this procedure, a balloon catheter is inserted into coronary blood vessels and
20 inflated at the site of the stenosis in an attempt to disrupt the atherosclerotic plaque and/or distend the disease-free area of the vessel to restore coronary blood flow. This procedure is conducted in approximately 300,000 patients a year in the United States. Although the procedure is highly successful at relieving the stenosis and restoring coronary blood flow, the utility of the procedure is
25 hampered by abrupt closure of the vessels in about 5% of patients (no re-flow phenomenon), and by restenosis in 30% to 40% of patients within three to six months of the procedure (Barry, W.L. and Sarembock, I.J., *Anticoag. Antiplatelet*

Ther. 12:517-535 (1994)). Restenosis can be described as an exaggerated form of vascular repair following arterial injury. New devices and a variety of pharmacological strategies have not been successful at eliminating this problem.

5 Numerous studies have been conducted in an attempt to elucidate the mechanism of restenosis following angioplasty. A large body of evidence is available that suggests that a neutrophil/platelet interaction is involved. The angioplasty procedure produces brief periods of myocardial ischemia. Current evidence suggests that activation of neutrophils during ischemia results in injury by: (a) the release of oxygen free radicals, proteolytic enzymes, and leukotoxin, 10 (b) aggregation and adherence of neutrophils to endothelial cells and subsequent capillary plugging and impairment of coronary blood flow, and (c) vasoconstriction that may result from leukotriene B₄ release by activated neutrophils. Platelets are activated by the release of oxygen free radicals, leukotrienes and platelet activating factor from activated neutrophils. Deposition 15 of platelets and thrombus formation have been shown to contribute to restenosis following angioplasty. The activated platelets release vasoconstrictor substances including thromboxane, serotonin and adenine nucleotides. In addition, platelets release platelet-derived growth factor which causes proliferation of vascular smooth muscle cells.

20 A primary stimulus for restenosis appears to be the activation of neutrophils in response to ischemia during the angioplasty procedure. Numerous clinical studies have shown an increased expression of surface adhesion molecules on neutrophils in blood taken from the coronary sinus following angioplasty (Mickelson, J.K., *et al.*, *JACC* 28:345-353 (1996); Ikeda, H., *et al.*, *Am. Heart* 25 *J.* 128:1091-1098 (1994); Neumann *et al.*, *Circulation* 92:748-755 (1995); Neumann *et al.*, *J. Am. Coll. Cardiol.* 27:819-824 (1996); Siminiak, T., *et al.*, *Br. Heart J.* 74:625-630 (1995); and Baj, Z., *et al.*, *Atherosclerosis* 106:159-168 (1994)). In addition, neutrophils taken from the coronary sinus following angioplasty show an increased release of toxic oxygen products and elastase when 30 tested *ex vivo* (Ikeda, H., *et al.*, *Am. Heart J.* 128:1091-1098 (1994)) and plasma

from the coronary sinus causes an activation of normal neutrophils *ex vivo* (Neumann, F-J., *et al.*, *Br. Heart J.* 70:27-34 (1993)).

Adenosine is an endogenous signal molecule that is released into the circulation during periods of ischemia. Adenosine exerts its physiological actions by activation of four subtypes of adenosine receptors designated A₁, A_{2A}, A_{2B} and A₃. Adenosine A_{2A} receptors are located on blood vessels where they mediate vasodilation (Ueda, M., *et al.*, *J. Med. Chem.* 34:1334-1339 (1991); Ueda, M., *et al.*, *J. Med. Chem.* 34:1340-1344 (1991); Niiya, K., *et al.*, *J. Med. Chem.* 35:4557-4561 (1992), Niiya, K., *et al.*, *J. Med. Chem.* 35:4562-4566 (1992); and Glover, D. K., *et al.*, *Circulation* 94:1726-1732 (1996)), on platelets where they mediate an inhibition of aggregation (Cristalli, G., *et al.*, *Arch. Pharmacol.* 349:644-650 (1994); and Varani, K., *et al.*, *Biochem. Pharmacol.* 48:1658-1661 (1994)) and on neutrophils where they mediate inhibition of adherence to endothelial cells and release of toxic oxygen products (Cronstein, B.N., *et al.*, *J. Clin. Invest.* 85:1150-1157 (1990), Cronstein, B.N., *et al.*, *J. Immunol.* 148:2201-2206 (1992); and Sullivan, G. W., *et al.*, *Int. J. Immunopharmac.* 17:793-803 (1995)). A compound that selectively activates only the A_{2A} adenosine receptors is expected to have anti-inflammatory actions in ischemic tissue by virtue of these three properties. Activation of A₁ receptors on neutrophils promotes chemotaxis and thereby increases migration of neutrophils to the site of injury. In addition, activation of A₁ receptors increases adherence of neutrophils to the endothelium. Adenosine's actions at the A₁ receptor are therefore pro-inflammatory (Cronstein, B.N., *et al.*, *J. Clin. Invest.* 85:1150-1157 (1990), and Cronstein, B.N., *et al.*, *J. Immunol.* 148:2201-2206 (1992)). Stimulation of A_{2A}-receptors and avoidance of A₁-receptor activation are the basis of this invention.

In vitro studies using human neutrophils have shown that A_{2A} selective agonists inhibit the release of oxygen free radicals and proteolytic enzymes from activated neutrophils and inhibit adherence of activated neutrophils to the endothelium (Cronstein, B. N., *et al.*, *J. Immunol.* 148:2201-2206 (1992), Cronstein, B.N., *et al.*, *J. Clin. Invest.* 85:1150-1157 (1990); and Sullivan, G. W.,

et al., *Int. J. Immunopharmac.* 17:793-803 (1995)). An A_{2A} selective agonist has also been shown to inhibit both the adhesion of activated canine neutrophils to canine cardiac myocytes and to reduce oxidative injury (Bullough, D. A., *et al.*, *J. Immunol.* 155:2579-2586 (1995)). Studies in pigs have shown that there is an increase in platelet deposition and neutrophil adhesion at the site of arterial injury produced by balloon inflation during angioplasty (Merhi, Y., *et al.*, *Circulation* 90:997-1002 (1994); and Provost, P. and Merhi, Y., *J. Pharmacol. Exp. Ther.* 277:17-21 (1996)).

Sollevi, in U.S. Pat. No. 5,449,655, discloses a method for percutaneous transluminal angioplasty comprising the concomitant continuous administration of adenosine to provide the beneficial properties of vasodilation, inhibition of platelet aggregation, and inhibition of presynaptic neural mechanisms regulating release of catecholamines.

Sollevi, in U.S. Pat. No. 5,534,504, discloses a method for coronary thrombolysis comprising the concomitant administration of adenosine with the thrombolytic agent to provide the above properties.

Bru-Magniez, *et al.*, in U.S. Pat. No. 5,459,132, discloses certain N⁶-substituted adenosine derivatives that are claimed as analgesics, anti-hypertensives and anti-proliferative agents used to treat cancer, psoriasis, atherosclerosis and restenosis phenomena.

Impaired blood flow to organs in mammals commonly results from occlusion of blood vessels as a result of atherosclerosis. Insufficient blood flow causes tissue ischemia and can result in morbidity such as myocardial infarction, stroke, or renal failure. Treatment options include invasive surgical procedures, such as angioplasty, atherectomy, or endarterectomy procedures, as well as the insertion of bypass grafts or stents to restore normal blood flow. A major limitation of the effectiveness of these treatments is that in a large proportion of subjects such cleared blood vessels can again become blocked within months.

Thus, the need continues to exist for methods and compositions that can be employed to reduce or prevent restenosis following an invasive revascularization procedure.

Summary of the Invention

5 A first aspect of the present invention concerns a method of reducing or preventing restenosis following a revascularization procedure in a mammal, comprising administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and an active agent selected from the group consisting of: (a) a selective adenosine A_{2A} receptor agonist, (b) the
10 combination of a selective adenosine A₁ antagonist and either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, and (c) a selective adenosine A₁ antagonist (in order to block the adenosine A₁ receptor activation by endogenously-released adenosine, while allowing for adenosine A_{2A} receptor activation by endogenously-released adenosine).

15 A second aspect of the present invention concerns compositions useful in the reduction and prevention of restenosis following revascularization. These compositions may comprise either: (a) a selective adenosine A_{2A} receptor agonist, (b) the combination of a selective adenosine A₁ antagonist and either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, or (c) a
20 selective adenosine A₁ antagonist (in order to block the adenosine A₁ receptor activation by endogenously-released adenosine, while allowing for adenosine A_{2A} receptor activation by endogenously-released adenosine).

25 A third aspect of the present invention provides for the use of one of the following for the manufacture of a medicament for reducing or preventing restenosis following revascularization in mammals: (a) a selective adenosine A_{2A} receptor agonist, (b) the combination of a selective adenosine A₁ antagonist and either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, or (c) a selective adenosine A₁ antagonist.

A fourth aspect of the present invention provides for a surgical process, comprising:

(i) administering to a subject a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and an active agent selected from the group consisting of: (a) a selective adenosine A_{2A} receptor agonist, (b) the combination of a selective adenosine A_1 antagonist and either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, and (c) a selective adenosine A_1 antagonist (in order to block the adenosine A_1 receptor activation by endogenously-released adenosine, while allowing for adenosine A_{2A} receptor activation by endogenously-released adenosine); and

(ii) practicing a revascularization procedure on said subject to remove an occlusion or to reroute blood flow through a bypass graft.

Detailed Description of the Preferred Embodiments

It has now been found that selective stimulation of adenosine A_{2A} receptors can reduce or prevent restenosis following revascularization. Adenosine A_{2A} receptors can be selectively stimulated by compositions including either: (a) a selective adenosine A_{2A} receptor agonist, (b) the combination of a selective adenosine A_1 antagonist and either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, or (c) a selective adenosine A_1 antagonist in order to block the adenosine A_1 receptor activation by endogenously-released adenosine are useful in the prevention and treatment of restenosis following revascularization.

A surgical procedure is provided, comprising:

(i) administering to a subject a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and an active agent selected from the group consisting of: (a) a selective adenosine A_{2A} receptor agonist, (b) the combination of a selective adenosine A_1 antagonist and either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, and (c) a selective

-7-

adenosine A₁ antagonist (in order to block the adenosine A₁ receptor activation by endogenously-released adenosine, while allowing for adenosine A_{2A} receptor activation by endogenously-released adenosine); and

5 (ii) practicing a revascularization procedure on said subject to remove an occlusion or to reroute blood flow through a bypass graft.

The procedure of the invention represents an improvement over the prior art in that the degree of restenosis is reduced.

10 A variety of revascularization procedures can be used, including percutaneous transluminal coronary angioplasty (PTCA), directional coronary atherectomy (DCA) and excimer laser angioplasty. In order to provide improved blood flow in the heart, insertion of coronary artery bypass grafts can be employed. PTCA is an alternative procedure that is also conducted in patients whose coronary blood flow is severely impaired by the presence of atherosclerotic plaques within the coronary blood vessels. In this procedure, a balloon catheter is inserted into coronary blood vessels and inflated at the site of the stenosis in an attempt to disrupt the atherosclerotic plaque and/or distend the disease-free area of the vessel to restore coronary blood flow.

15 In the present invention, a method of reducing or preventing restenosis following revascularization procedures is provided wherein a mammal, preferably a human, is administered an effective amount of either: (a) a selective adenosine A_{2A} receptor agonist, (b) the combination of a selective adenosine A₁ antagonist and either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, or (c) a selective adenosine A₁ antagonist in order to block the adenosine A₁ receptor activation by endogenously-released adenosine. A composition including one of (a), (b) or (c) is administered starting at a selected time prior to the revascularization procedure and is administered in an effective amount such that restenosis is reduced or prevented. Administration is preferably continued following the procedure for a period of up to two days.

25 Preferably, the compositions and methods of the present invention employ 30 a selective adenosine A_{2A} receptor agonist.

The term "selective stimulation of adenosine A_{2A} receptors" refers to a substantially greater stimulation of adenosine A_{2A} receptors compared to adenosine A₁ receptors. Selective stimulation occurs when the ratio of A_{2A} : A₁ stimulation for a particular agent or combination of agents is greater than the A_{2A} : A₁ stimulation ratio obtained when adenosine alone is employed as the agent.

The term "effective amount" refers either to: (a) a concentration of an adenosine A_{2A} receptor agonist, (b) a concentration of an adenosine A₁ receptor antagonist in combination with a concentration of either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine agonist, or (c) a concentration of a selective adenosine A₁ antagonist (to counteract the adenosine A₁ receptor activation by endogenously released adenosine) that is sufficient to allow selective adenosine A_{2A} receptor activation by endogenously released adenosine in response to the induced ischemia from the revascularization procedure.

The term "selective adenosine A_{2A} receptor agonist" refers to agonists that stimulate preferentially the adenosine A_{2A} receptor and do not stimulate substantially the adenosine A₁ receptor. Compounds may be chosen as selective A_{2A} agonists by testing for cardiovascular activity as described in Niiya, K., *et al.*, *J. Med. Chem.* 35:4557-4561 (1992) and demonstrating an A₁/A₂ selectivity ratio therein defined as greater than approximately 100. As will be appreciated by one of ordinary skill in the art, other assays can be employed to screen for adenosine A_{2A} receptor agonism.

Examples of selective adenosine A_{2A} receptor agonists include: 2-(substituted amino)adenosine 5'-carboxamides, described in U.S. Patent No. 4,968,697; 2-(substituted amino)adenosines, described in U.S. Patent No. 5,034,381; imidazo-[4,5-b]-pyridine derivatives, described in U.S. Patent No. 4,977,144; and 2-(substituted alkynyl)adenosines, described in U.S. Patent No. 5,189,027. Additional examples of selective A_{2A} receptor agonists include 2-hydrazoadenosines, described in U.S. Patent No. 5,278,150 and 2-aralkoxy and 2-alkoxy adenosines, described in U.S. Patent No. 5,140,015.

Examples of agonists selective for adenosine A_{2A} receptors include, but are not limited to, 2-cyclohexylmethylenedrazinoadenosine, 2-(3-cyclohexenyl)methylenedrazinoadenosine, 2-isopropylmethylenedrazinoadenosine, N-ethyl-1'-deoxy-1'-[6-amino-2-[(2-thiazolyl)ethynyl]-9H-purin-9-yl]-β-D-ribofuranuronamide, N-ethyl-1'-deoxy-1'-[6-amino-2-[hexynyl]-9H-purin-9-yl]-β-D-ribofuranuronamide, 2-(1-hexyn-1-yl)adenosine-5'-N-methyluronamide, 5'-chloro-5'-deoxy-2-(1-hexyn-1-yl)adenosine, N⁶-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)adenosine, 2-(2-phenyl)ethoxyadenosine, 2-[2-(4-methylphenyl)-ethoxy]adenosine, 2-[2-(4-fluorophenyl)ethoxy]adenosine, 2-(2-(2-naphthyl)ethoxy)adenosine, 2-[p-(2-carboxyethyl)phenethylamino-5'-N-ethyl-carboxamidoadenosine (CGS-21680), 2-(2-cyclohexyl)ethoxyadenosine, 2-octynyladenosine (YT-146), 2-thiazolylethynyladenosine and 2-phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS-21577).

Preferred selective A_{2A} agonists include 2-cyclohexylmethylenedrazinoadenosine, 2-(3-cyclohexenyl)methylenedrazinoadenosine, 2-isopropylmethylenedrazinoadenosine, 2-(2-phenyl)ethoxyadenosine, 2-(2-(4-methylphenyl)ethoxyadenosine, 2-(2-cyclohexyl)ethoxyadenosine, and 2-(2-(p-carboxyethyl)phenyl)ethylamino-5'-N-ethyl-carboxamidoadenosine.

The term "selective adenosine A₁ receptor antagonist" refers to antagonists that bind preferentially to the adenosine A₁ receptor and do not bind substantially to adenosine A_{2A} or A_{2B} receptors. Compounds may be chosen as selective A₁ antagonists by screening for cardiovascular activity as described in Belardinelli, *et al.*, *J. Pharm. Exp. Ther.* 275:1167-1176 (1995)), and demonstrating that the compound produces a statistically significantly (vs. control) greater blockade of the A₁ receptor mediated increase in S-H interval as compared with the blockade of the A₂ receptor mediated increase in coronary conductance. As will be appreciated by one of ordinary skill in the art, different assays can be employed to screen for A₁ selective antagonism.

Examples of selective adenosine A₁ receptor antagonists include: [[2,3,7,9-tetrahydro-1,3-dialkyl-2,6-dioxo-1H-purin-8-yl]alkyl]phenyl

heteroalkanoic acids and esters thereof, described in U.S. Patent No. 5,208,240; 1,3-dialkyl-8-(substituted phenyl)xanthines, described in U.S. Patent No. 4,783,530; epoxides of 1,3-dialkylxanthines and adenosines, described in U.S. Patent No. 5,446,046; 8-tricycloalkyl substituted 1,3-dialkylxanthines, described in U.S. Patent No. 5,395,836; and 1,3-dialicyclicalkyl-8-substituted xanthines. Additional examples of compounds that act as selective A₁ receptor antagonists include N⁶-substituted 9-methyladenines as described in U.S. Patent No. 5,066,655, published International application WO 96/06845, and U.S. Appl. No. 08,299,992.

Examples of antagonists selective for adenosine A₁ receptors include, but are not limited to, (±)-N⁶-endonorboman-2-yl-9-methyladenine (N-0861), (±)-N⁶-[endo-2'-norbomyl]-8-(isopropylmethylamino)-9-methyladenine, (±)-N⁶-[endo-2'-(endo-5'-hydroxy)-norbomyl]-8-(isopropylmethylamino)-9-methyladenine, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), xanthine amine congener (XAC), 8-(noradamantan-3-yl)-1,3-dipropylxanthine (NAX), 8-(cyclopentan-3-one)-1,3-dipropylxanthine (KFM19), 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (KF-15372), (R)-7,8-dimethyl-2-phenyl-9-(1-phenylethyl)-7-deazaadenine, 1-propyl-3-(4-amino-3-iodophenylethyl)-8-cyclopentylxanthine (BW-A844U), (+)-(R)-[(E)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)acryloyl]-2-piperidine ethanol (FK453), 7-[2-[ethyl(2-hydroxyethyl)amino]ethyl]-3,7-dihydro-1,3-dimethyl-8-(phenylmethyl)-1H-purine-2,6-dione (bamiphylline), 1,3-dipropyl-8-sulfophenylxanthine (DPSPX), 1,3-dipropyl-8-[2-(5,6-exo-epoxy)-(1S,2S)-norbom-2-yl]xanthine (CVT-124), and 7,8-dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1H-imidazo[2,1-i]purin-5(4H)-one.

The term "non-selective adenosine agonist" refers to agonists that bind to adenosine A₁, A_{2A}, and A_{2B} receptors. Compounds may be chosen as non-selective agonists by testing for cardiovascular activity as described in Ueda, M., *et al.*, *J. Med. Chem.* 34:1334-1339 (1991) and demonstrating an A₁/A₂ selectivity ratio therein defined between about 5 and about 100. Examples of non-selective

-11-

agonists include, but are not limited to, adenosine, 5'-N-ethylcarboxamidoadenosine (NECA), and 2-chloroadenosine.

The present invention provides for the reduction or prevention of restenosis following activation of neutrophils by selectively activating adenosine A_{2A} receptors during revascularization procedures. The present method will not only greatly reduce or prevent ischemic injury due to adherence and release of toxic oxygen products from neutrophils, but will also inhibit the subsequent activation of platelets that leads to the no-reflow phenomenon and restenosis.

The no-reflow phenomenon that occurs in about 5% of patients undergoing PTCA is believed to be due to aggregation of platelets and neutrophils, which causes a blockage of blood flow within the vessels and vasoconstriction from substances released from the platelets. The acute complication rate following PTCA can be reduced by aspirin, which inhibits platelet aggregation. Consequently, aspirin is routinely administered during angioplasty procedures. Aspirin, however, has been shown to have very little effect on the delayed complication rate of restenosis. In addition, aspirin can inhibit the production of vasodilatory prostaglandins and thereby exacerbate vasospasm that can occur following PTCA. In contrast, the present invention is superior to aspirin in reducing acute blood vessel closure following angioplasty procedures by producing a direct inhibition of platelet aggregation (Cristalli, G., *et al.*, *Arch. Pharmacol.* 349:644-650 (1994); and Varani, K., *et al.*, *Biochem. Pharmacol.* 48:1658-1661 (1994)) while having the added benefit of causing vasodilation.

Although deposition of platelets and thrombus formation have been demonstrated to contribute to restenosis following angioplasty, antiplatelet or antithrombotic therapy have generally been demonstrated to have little effect on restenosis after balloon angioplasty (Barry, W. L. and Sarembock, I. J., *Anticoag. Antiplatelet Ther.* 12:517-535 (1994)). This may partly be due to the fact that all of the agents tested to date in humans are relatively weak and non-specific inhibitors of platelet function. Also, restenosis is a multifactorial effect and

-12-

blocking the action of the platelets alone may not be sufficient to inhibit restenosis. The present invention provides a method to prevent ischemic injury and restenosis as a result of inhibition of both neutrophil activation and platelet activation.

5 In the present invention, compositions are also provided that are useful in the reduction or prevention of restenosis following revascularization procedures in a mammal, preferably a human. Such compositions comprise either: (a) a selective adenosine A_{2A} receptor agonist, (b) the combination of a selective adenosine A_1 antagonist and a non-selective adenosine agonist, or (c) a selective adenosine A_1 antagonist in order to block the adenosine A_1 receptor activation by
10 endogenously-released adenosine in response to the induced ischemia from the revascularization procedure.

In addition to the pharmacologically active compounds, the pharmaceutical compositions can contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into
15 preparations that can be used pharmaceutically. Pharmaceutically acceptable carriers include, but are not limited to, saline, water or an aqueous dextrose solution, Molecusol™ or similar sugar solution, combinations of ethanol and aqueous buffer solutions that are commonly used in the art (for example, phosphate buffers), and dilute sodium hydroxide (approx. 0.2 N) solutions.
20 Suitable lipophilic solvents or vehicles include synthetic fatty acid esters, for example, ethyl oleate or triglycerides or polyethylene glycol 400. As will be appreciated, various carrier and/or excipients may be called for depending upon the solubility of the particular agent employed.

25 The dosage of selective A_{2A} agonist administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any and potency of the particular agonist. Dosages for many known A_{2A} agonists are described in the prior art. Optimized doses can be determined by one or ordinary skill in the art by routine experimentation.

30 The ratio of an adenosine A_1 antagonist and either a selective adenosine A_2 agonist or a nonselective agonist will depend on the potencies of the individual

agents. However, the combination should be selected such that its A_1/A_2 selectivity ratio is greater than approximately 100 as defined in Niiya, K., *et al.*, *J. Med. Chem.*, 35:4557-4561 (1992). Adjusting the concentrations of each agent based upon their relative potencies to arrive at the required selective stimulation of A_{2A} receptors can be accomplished by the artisan of ordinary skill employing routine experimentation.

It is preferred that compositions of the present invention be administered prior to revascularization procedures, preferably 30 minutes prior to performing revascularization procedure, and then be continuously administered for up to 2 days, preferably for 1 day after the revascularization procedure, preferably by continuous infusion. However, repeated bolus injections can be employed.

It is preferred that these compositions be administered by intravenous bolus injection or infusion directly to the vasculature involved in these revascularization procedures.

The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered and obvious to those skilled in the art are within the spirit and scope of the invention.

Example 1

Reduction of Restenosis by Administering an Adenosine A_{2A} Selective Agonist

Focal femoral atherosclerosis is induced in rabbits by air desiccation endothelial injury followed by a 2% cholesterol, 6% peanut oil diet for one month. The degree of arterial stenosis is assessed angiographically. At this time, balloon angioplasty is performed using a 2.5 mm balloon, which is inserted into the region of stenosis and inflated with three 60-second, 10-atmosphere inflations 60 seconds apart. This procedure is similar to that used in humans for clearing coronary blood vessels. Rabbits receive an intravenous infusion of

-14-

2-cyclohexylmethylenhydrazinoadenosine (0.6 µg/kg/min) or vehicle starting 10 minutes before the angioplasty procedure and continuing for 1 day following angioplasty. Angiograms are performed before and after angioplasty and at 28 days after angioplasty. These angiograms are analyzed quantitatively for decrease of arterial stenosis. Rabbits are sacrificed 28 days following angioplasty and arteries are analyzed histopathologically and morphometrically. The degree of arterial stenosis in the rabbit group treated with the adenosine A_{2A} selective agonist is significantly less than the arterial stenosis in the rabbit group treated with vehicle.

Example 2

Reduction of Restenosis by Co-Administration of an Adenosine A₁ Antagonist and a Non-Selective Adenosine Agonist

Example 1 is followed, with the exception that one rabbit group is treated with an effective amount of a combination of (±)-N⁶-endonorboman-2-yl-9-methyladenine (0.25 mg/kg/min) and 5'-N-ethylcarboxamidoadenosine (6 µg/kg/min) instead of 2-cyclohexylmethylenhydrazinoadenosine. The degree of arterial stenosis in the rabbit group treated with the combination of a selective adenosine A₁ antagonist ((±)-N⁶-endonorboman-2-yl-9-methyladenine) and a non-selective adenosine agonist (5'-N-ethylcarboxamidoadenosine) is significantly less than the arterial stenosis in the rabbit group treated with vehicle.

Example 3

Reduction of Restenosis by Co-Administration of an Adenosine A₁ Antagonist and an Adenosine A_{2A} Selective Agonist

Example 1 is followed, with the exception that one rabbit group is treated with an effective amount of a combination of (±)-N⁶-endonorboman-2-yl-9-methyladenine (0.25 mg/kg/min) and 2-cyclohexylmethylenhydrazinoadenosine (0.6 µg/kg/min) instead of 2-cyclohexylmethylenhydrazinoadenosine alone. The

-15-

degree of arterial stenosis in the rabbit group treated with this combination is significantly less than the arterial stenosis in the rabbit group treated with vehicle.

Example 4

Reduction of Restenosis by Administering a Selective Adenosine A₁ Antagonist

Example 1 is followed, with the exception that the non-vehicle rabbit group is not treated with 2-cyclohexylmethylethyldrazinoadenosine, but with an effective amount of (\pm) -N⁶-endonorbonan-2-yl-9-methyladenine (0.25 µg/kg/min) in order to block the adenosine A₁ receptor activation by endogenously released adenosine in response to the induced ischemia from the revascularization procedure. The degree of arterial stenosis in the rabbit group treated with the selective adenosine A₁ antagonist ((\pm) -N⁶-endonorbonan-2-yl-9-methyladenine) is significantly less than the arterial stenosis in the rabbit group treated with vehicle.

Example 5

In Vivo Inhibition of Neutrophil and Platelet Activation by Administering an Adenosine A_{2A} Selective Agonist

Humans are subjected to balloon angioplasty, and receive either an intravenous infusion of 2-cyclohexylmethylethyldrazinoadenosine (0.6 µg/kg/min) or vehicle starting 10 minutes before the angioplasty procedure and continuing for 1 day following angioplasty. *In vivo* activation of neutrophils and platelets are measured by taking a blood sample from the coronary sinus and measuring the expression of surface adhesion molecules on platelets and neutrophils by flow cytometry. The group receiving 2-cyclohexylmethylethyldrazinoadenosine demonstrates significantly fewer platelets and neutrophils that express surface adhesion molecules than the group receiving vehicle.

-16-

Example 6***Reduction of Restenosis by Administering
an Adenosine A_{2A} Selective Agonist***

5 Humans afflicted with impaired blood flow due to stenotic arteries are
subjected to balloon angioplasty, and receive either an intravenous infusion of 2-
cyclohexylmethylenhydrazinoadenosine (0.6 µg/kg/min) or vehicle starting 10
minutes before the angioplasty procedure and continuing for 1 day following
angioplasty. Six months following the balloon angioplasty procedure, angiography
is performed on these subjects to examine the blood vessel subjected to the
10 angioplasty. The incidence of restenosis is significantly less in the group receiving
2-cyclohexylmethylenhydrazinoadenosine than the group receiving vehicle.

15 Having now fully described this invention, it will be understood to those
of ordinary skill in the art that the same can be performed within a wide and
equivalent range of conditions, formulations, and other parameters without
affecting the scope of the invention or any embodiment thereof. All patents,
patent applications, and publications cited herein are fully incorporated by
reference herein in their entirety.

What Is Claimed Is:

1. A method of reducing or preventing restenosis that occurs following a revascularization procedure, comprising:

5 administering to a mammal in need of such treatment a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and an active agent selected from the group consisting of:

(a) a selective adenosine A_{2A} receptor agonist,
(b) the combination of a selective adenosine A₁ antagonist and either a selective adenosine A_{2A} receptor agonist or a non-selective adenosine
10 agonist, and

(c) a selective adenosine A₁ antagonist in order to block the adenosine A₁ receptor activation by endogenously released adenosine;

wherein said active agent is administered starting at a selected time prior to a revascularization procedure and is administered in an effective amount such that
15 restenosis is reduced or prevented.

2. The method of claim 1, wherein said pharmaceutical composition is administered beginning 30 minutes prior to said revascularization procedure.

3. The method of claim 2, wherein said pharmaceutical composition is administered continuously for up to two days after said revascularization
20 procedure.

4. The method of claim 1, wherein said pharmaceutical composition is administered by intravenous bolus injection or by infusion directly to the vasculature being treated by said revascularization procedure.

5. The method of claim 1, wherein the revascularization procedure is performed on one or more of coronary arteries, carotid arteries, renal arteries, or leg arteries.

6. The method of claim 1, wherein the revascularization procedure is one of balloon angioplasty, insertion of a bypass graft, insertion of a stent, percutaneous transluminal coronary angioplasty, or directional coronary atherectomy.

7. The method of claim 1, wherein the revascularization procedure involves excimer laser angioplasty.

8. The method of claim 1, wherein the mammal is a human.

9. The method of claim 1, wherein the agent is a selective A_{2A} receptor agonist.

10. The method of claim 9, wherein the selective A_{2A} receptor agonist is selected from the group consisting of:

2-cyclohexylmethylenedrazinoadenosine,

2-(3-cyclohexenyl)methylenedrazinoadenosine,

2-isopropylmethylenedrazinoadenosine, N-ethyl-1'-deoxy-1'-(6-amino-2-((2-thiazolyl)ethynyl)-9H-purin-9-yl)- β -D-ribofuranuronamide,

N-ethyl-1'-deoxy-1'-(6-amino-2-(hexynyl)-9H-purin-9-yl)- β -D-ribofuranuronamide,

2-(1-hexyn-1-yl)adenosine-5'-N-methyluronamide,

5'-chloro-5'-deoxy-2-(1-hexyn-1-yl)adenosine,

N⁶-(2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)adenosine,

2-(2-phenyl)ethoxyadenosine,

2-(2-(4-methylphenyl)ethoxy)adenosine,

2-(2-(4-fluorophenyl)ethoxy)adenosine,
2-(2-(2-naphthyl)ethoxy)adenosine,
2-(*p*-(2-carboxyethyl)phenyl)ethylamino-5'-N-ethyl-carboxamidoadenosine,
2-(2-cyclohexyl)ethoxyadenosine,
5 2-octynyladenosine,
2-thiazolylethynyladenosine, and
2-phenethylamino-5'-N-ethylcarboxamidoadenosine.

11. The method of claim 10, wherein said selective A_{2A} agonist is 2-cyclohexylmethylenedihydrazinoadenosine, 2-(3-cyclohexenyl)methylene-
10 hydrazinoadenosine, 2-isopropylmethylenedihydrazinoadenosine, 2-(2-phenyl)ethoxyadenosine, 2-(2-(4-methylphenyl)ethoxyadenosine, 2-(2-cyclohexyl)ethoxyadenosine, or 2-(2-(*p*-carboxyethyl)phenyl)ethylamino-5'-N-ethyl-carboxamidoadenosine.

12. The method of claim 1, wherein said agent is a selective A₁
15 receptor antagonist.

13. The method of claim 12, wherein said agent is a selective A₁ receptor antagonist selected from the group consisting of:

(±)-endonorboman-2-yl-9-methyladenine,
(±)-N⁶-[endo-2'-norbornyl]-8-(isopropylmethylamino)-9-methyladenine,
20 (±)-N⁶-[endo-2'-(endo-5'-hydroxy)-norbornyl]-8-(isopropylmethylamino)-9-methyladenine,
8-cyclopentyl-1,3-dipropylxanthine,
xanthine amine congener,
8-(noradamantan-3-yl)-1,3-dipropylxanthine,
25 8-(cyclopentan-3-one)-1,3-dipropylxanthine,
8-(dicyclopropylmethyl)-1,3-dipropylxanthine,
(R)-7,8-dimethyl-2-phenyl-9-(1-phenylethyl)-7-deazaadenine,

-20-

1-propyl-3-(4-amino-3-iodophenylethyl)-8-cyclopentylxanthine,
(+)-(R)-[(E)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)acryloyl]-2-piperidine
ethanol,
7-[2-[ethyl(2-hydroxyethyl)amino]ethyl]-3,7-dihydro-
1,3-dimethyl-8-(phenylmethyl)-1H-purine-2,6-dione,
1,3-dipropyl-8-sulfophenylxanthine,
1,3-dipropyl-8-[2-(5,6-exo-epoxy)-(1S,2S)-norborn-2-yl]xanthine, and
7,8-dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1H-imidazo[2,1-i]purin-5(4H)-
one.

14. The method of claim 12, wherein said agent further comprises a
non-selective adenosine agonist selected from the group consisting of adenosine,
5'-N-ethylcarboxamidoadenosine and 2-chloroadenosine.

15. A method of providing revascularization in a subject, comprising:

(i) administering to a subject a pharmaceutical composition comprising
a pharmaceutically acceptable carrier or diluent, and
an active agent selected from the group consisting of:

- (a) a selective adenosine A_{2A} receptor agonist,
- (b) the combination of a selective adenosine A_1 antagonist
and either a selective adenosine A_{2A} receptor agonist or a
non-selective adenosine agonist, and
- (c) a selective adenosine A_1 antagonist (in order to block
the adenosine A_1 receptor activation by endogenously-
released adenosine, while allowing for adenosine A_{2A}
receptor activation by endogenously-released adenosine);
and

(ii) practicing a revascularization procedure on said subject to remove
an occlusion or to reroute blood flow through a bypass graft.

-21-

16. The method of claim 15, wherein said pharmaceutical composition is administered beginning 30 minutes prior to said revascularization procedure.

17. The method of claim 16, wherein said pharmaceutical composition is administered continuously for up to two days after said revascularization procedure.

18. The method of claim 15, wherein said pharmaceutical composition is administered by intravenous bolus injection or by infusion directly to the vasculature being treated by said revascularization procedure.

19. The method of claim 15, wherein the revascularization procedure is performed on one or more of coronary arteries, carotid arteries, renal arteries, or leg arteries.

20. The method of claim 15, wherein the revascularization procedure is one of balloon angioplasty, insertion of a bypass graft, insertion of a stent, percutaneous transluminal coronary angioplasty, or directional coronary atherectomy.

21. The method of claim 15, wherein the revascularization procedure involves excimer laser angioplasty.

22. The method of claim 15, wherein the agent is a selective A_{2A} receptor agonist.

23. The method of claim 22, wherein the selective A_{2A} receptor agonist is selected from the group consisting of:
2-cyclohexylmethylenhydrazinoadenosine,
2-(3-cyclohexenyl)methylenhydrazinoadenosine,

2-isopropylmethylenhydrazinoadenosine, N-ethyl-1'-deoxy-1'-(6-amino-2-((2-thiazolyl)ethynyl)-9H-purin-9-yl)- β -D-ribofuranuronamide,
 N-ethyl-1'-deoxy-1'-(6-amino-2-(hexynyl)-9H-purin-9-yl)- β -D-ribofuranuronamide,
 5 2-(1-hexyn-1-yl)adenosine-5'-N-methyluronamide,
 5'-chloro-5'-deoxy-2-(1-hexyn-1-yl)adenosine,
 N⁶-(2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)adenosine,
 2-(2-phenyl)ethoxyadenosine,
 2-(2-(4-methylphenyl)ethoxy)adenosine,
 10 2-(2-(4-fluorophenyl)ethoxy)adenosine,
 2-(2-(2-naphthyl)ethoxy)adenosine,
 2-(*p*-(2-carboxyethyl)phenyl)ethylamino-5'-N-ethyl-carboxamidoadenosine,
 2-(2-cyclohexyl)ethoxyadenosine,
 2-octynyladenosine,
 15 2-thiazolylethynyladenosine, and
 2-phenethylamino-5'-N-ethylcarboxamidoadenosine.

24. The method of claim 15, wherein said agent is a selective A₁ receptor antagonist.

25. The method of claim 24, wherein said agent is a selective A₁ receptor antagonist selected from the group consisting of:

(±)-endonorboman-2-yl-9-methyladenine,
 (±)-N⁶-[endo-2'-norbonyl]-8-(isopropylmethylamino)-9-methyladenine,
 (±)-N⁶-[endo-2'-(endo-5'-hydroxy)-norbonyl]-8-(isopropylmethylamino)-9-methyladenine,
 25 8-cyclopentyl-1,3-dipropylxanthine,
 xanthine amine congener,
 8-(noradamantan-3-yl)-1,3-dipropylxanthine,
 8-(cyclopentan-3-one)-1,3-dipropylxanthine,

-23-

8-(dicyclopropylmethyl)-1,3-dipropylxanthine,
(R)-7,8-dimethyl-2-phenyl-9-(1-phenylethyl)-7-deazaadenine,
1-propyl-3-(4-amino-3-iodophenylethyl)-8-cyclopentylxanthine,
(+)-(R)-[(E)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)acryloyl]-2-piperidine
5 ethanol,
7-[2-[ethyl(2-hydroxyethyl)amino]ethyl]-3,7-dihydro-
1,3-dimethyl-8-(phenylmethyl)-1H-purine-2,6-dione,
1,3-dipropyl-8-sulfophenylxanthine,
1,3-dipropyl-8-[2-(5,6-exo-epoxy)-(1S,2S)-norborn-2-yl]xanthine, and
10 7,8-dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1H-imidazo[2,1-i]purin-5(4H)-
one.

26. The method of claim 25, wherein said agent further comprises a non-selective adenosine agonist selected from the group consisting of adenosine, 5'-N-ethylcarboxamidoadenosine and 2-chloroadenosine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/12717

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/70, 31/505

US CL :514/46, 263, 267

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/46, 263, 267

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

search terms: restenosis and adenosine?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,534,504 A (SOLLEVI) 09 July 1996, column 1, lines 54-59 and column 4, line 46 to column 22, line 25.	1-26
A	US 5,459,132 A (BRU-MAGNIEZ et al.) 17 October 1995, entire document.	1-26

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

14 SEPTEMBER 1998

Date of mailing of the international search report

OCT 13 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

THEODORE J. CRIARES

Telephone No. (703) 308-1235